Isozyme Variations in Human Malignant Melanoma


Departments of Obstetrics and Gynecology [R. P., D. M. M.] and Urology [R. P.], Baylor College of Medicine; Departments of Surgery [M. M. R.], Pediatrics [D. M. M.], and Anatomical Pathology [J. L. S.]; and Section of Medical Genetics [C. R. S.], M. D. Anderson Hospital and Tumor Institute, The University of Texas, Houston, Texas 77025

SUMMARY

Eleven enzymes were studied in human malignant melanoma tissue by starch gel electrophoresis, followed by zymogram staining. The results indicate that the frequency of altered enzymes is quite high in this tumor. In a total of 21 human malignant melanoma tumor samples, 6 of 9 different electrophoretically detectable enzymes studied showed alteration in at least 1 or more tumor samples. These preliminary data suggest that human malignant melanoma undergoes genetic or epigenetic changes. The alteration in some enzymes, such as 6-phosphogluconate dehydrogenase, could prove to be of diagnostic value.

INTRODUCTION

The isozyme patterns of various enzymes in experimental and human tumors are known to be different from those of normal tissues. This has been comprehensively reviewed by Criss (6). From these observations, it is speculated that cancer cells are characterized by a disordered expression of many genes (13, 14). Experimental studies on the genetic and hereditary aspects of melanoma in certain fish, Drosophila, the Mexican axolotl, and mice, as well as spontaneous occurrence of melanoma in horses, swine, and reptiles, show the influence of hereditary factors on the occurrence of malignant melanoma in these animals (5, 7, 18). As far as human melanoma is concerned, hereditary and genetic information is reported only on the basis of family pedigree studies (2, 3, 9-12, 19).

Therefore, it seemed important to see whether human malignant melanoma showed changes in enzymes and isozyme patterns and, if so, whether these changes could be of any significance in defining the biochemical and genetic nature of this disease.

MATERIALS AND METHODS

Twenty-one human malignant melanoma specimens were obtained at operation at M. D. Anderson Hospital and Tumor Institute, Houston, Texas. Blood samples as well as normal muscle and skin tissue were obtained from these patients. Sera were separated from the blood cells for electrophoretic studies. Extracts of all the tissues were prepared as previously described (16). Tissues were first washed twice in cold 0.9% NaCl solution containing 1.5 x 10^{-3} M disodium EDTA, followed by several washes in ice-cold deionized water. Tissues were then homogenized in 1:2 w/v deionized water, and supernatant was collected after centrifugation for 15 to 20 min at 10,000 x g.

Electrophoresis of the supernatants and sera samples was done on vertical starch gels with the use of buffer systems (Table 1). Gel slices were stained for the dehydrogenases of lactate, malate, \( \alpha \)-glycerophosphate, 6-phosphogluconate, glucose 6-phosphate, glyceraldehyde 3-phosphate, and isocitrate, and for \( \alpha \)-esterases, alkaline phosphatases, phosphoglucomutases, and aldolases. The staining methods and buffer systems used were as described by Shaw and Prasad (17). The protein fractions of sera and tissue were studied after electrophoresis by staining with 1% brilliant blue in 7.5% acetic acid.

RESULTS

Table 1 shows the morphology and histology of tumor tissues from different patients.

As shown in Table 2, only the 1st 9 enzymes studied were expressed in tumor tissues. \( \alpha \)-Glycerocephosphate dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase were present only in the muscle tissue. Phosphoglucomutase was present only in the tumor tissue homogenates. No variation was found in the lactate and malate dehydrogenase patterns of the tumor tissues (Charts 1 and 2). Five bands of lactate dehydrogenase were expressed in all of the tumor tissue, as well as in all skin and muscle tissues. The relative activities of isozymes themselves, however, differed from tumor to tumor. In some tumors there was more activity of lactate dehydrogenase 1 and lactate dehydrogenase 2. In some, all of the bands were of essentially equal intensity. In all of the tumor tissue homogenates, both the supernatant and the mitochondrial-type malate dehydrogenases were expressed (Chart 2). There was no variation in the pattern of this enzyme, except that some tumors had quite high activity compared to others and that, in 1 tumor sample from Patient 6, the \( m \)-malate dehydrogenase had a very distinct subband, which is absent in all the other tumor and muscle tissues. There was little variation in the \( \alpha \)-esterase patterns of the tumor tissue homogenates (Chart 3). One tumor...
sample from Patient 1 had only 1 band of this enzyme. The remainder of the tumors had multiple bands (both cathodal and anodal), but the electrophoretic mobility of these bands varied. Isocitrate dehydrogenase activity was absent in tumor samples from Patients 1 and 7 (Chart 4). In most of the samples, the enzyme was expressed as a single anodal band. However, tumors from Patients 3, 10, and 11 had 2 such bands and, in 2 other tumor homogenates (Patients 4 and 5), very faint cathodal bands were present. 6-Phosphogluconate dehydrogenase activity was present in all but 1 tumor sample (from Patient 1). Of these, 18 had fast-moving bands identical to skin and 2 had slow-moving bands identical to muscle tissue (Charts 5 and 6).

Glucose 6-phosphate dehydrogenase, alkaline phosphatase, and phosphoglucomutase bands were not distinctive, but their patterns were variable. Protein stains showed that tumor tissues have fewer bands than do sera, muscle, and skin tissues. All the enzymes mentioned above were also studied in sera samples. No variation of these enzymes was found in the sera samples of the melanoma patients.
Isozyme Variations in Malignant Melanoma

Chart 1. Ten representative patterns of lactate dehydrogenase from human malignant melanoma tissues (O, origin). The numbers of the tumor samples correspond to different patients: m, muscle; s, skin. All remaining tumor samples had same type of pattern as shown in this chart.

Chart 2. Six representative patterns of malate dehydrogenase from human malignant melanoma tissues (O, origin). The numbers of tumor samples correspond to different patients: m, muscle tissue; s, skin tissue.

Chart 3. Eight representative patterns of α-esterase from human malignant melanoma tissues (O, origin). The numbers of the tumor samples correspond to different patients. Five tumor samples (from Patients 5, 12, 14, 18, and 19) had patterns identical to tumor bands of Patient 2; 5 tumors (from patients 4, 6, 9, 13, and 16) had identical patterns to tumor bands of Patient 3; and tumor samples from Patients 11, 17, and 21 had identical patterns to tumor bands from Patient 7. Muscle and skin tissues had bands similar to that of Patient 3.

Chart 4. Six representative patterns of isocitrate dehydrogenase from human malignant melanoma tissue (O, origin). The numbers of tumor samples correspond to the different patients: m, muscle tissue. All the remaining tumor tissues had bands identical to either Patient 2 or 9. In tumor tissues of Patients 1 and 7, isocitrate dehydrogenase was absent. Skin tissue had similar bands as the muscle tissue.

Chart 5. Seven representative patterns of 6-phosphogluconate dehydrogenase (O, origin). The numbers of tumor samples correspond to different patients. All remaining samples had fast moving bands such as Patients 3, 6, 7, and 9.

Chart 6. 6-Phosphogluconate dehydrogenase patterns of human malignant melanoma tissues (O, origin). The number of the tumor samples correspond to different patients; s, skin tissue; m, muscle tissue.
DISCUSSION

It is clear from the results that the frequency of altered enzymes is quite high in human malignant melanoma. In a total of 21 tumor samples and 9 different enzymes that were electrophoretically detectable in tumor tissues, 6 showed alteration in at least one or more tumor samples. Lactate and malate dehydrogenases were the only enzymes that remained consistently unaltered in all of the tumor tissues studied. The unequal activity of the lactate dehydrogenases, which is clearly indicated by density (or intensity) of the bands, occurs probably because the tumors were taken from different parts (tissues) of the body. In many malignant tumors, lactate dehydrogenase has a significant correlation with the growth rate of the malignant tumors (1, 4, 20). In general, preponderance of muscle-type lactate dehydrogenase is presumed to be related to higher glycolytic capacity of the cells. It has been also shown that this muscle-type lactate dehydrogenase is present in excess over heart-type lactate dehydrogenase in tumor types that require anaerobic respiration for their neoplastic development (15). Thus, the altered distribution of lactate dehydrogenase in melanoma tissue may suggest that this tumorigenesis does not require anaerobic respiration for its development in vivo. The absence of glyceraldehyde 3-phosphate dehydrogenase and α-glycerophosphate dehydrogenase in the tumors also supports this speculation. The significance of altered patterns of α-esterase, alkaline phosphatase, isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase, glucose 6-phosphate dehydrogenase, and phosphoglucomutase is not apparent at this time. If a tumor has one altered enzyme, it does not necessarily have other altered enzymes. The alteration of some of these enzymes may be explained by the fact that individual patients have specific tumor types. Lewis et al. (8) have reported tumor-specific cytotoxic autoantibodies in human malignant melanoma. They found that the effective antibody activity was specific for the individual patients.

The 6-phosphogluconate dehydrogenase pattern of most tumors and the skin tissues have similar bands (fast-moving), while muscle tissues have slower moving bands. It would be of interest to study a large number of tumor tissues along with skin biopsies of the same patients and compare them with the skin tissues of normal individuals. If there is a consistency of the fast-moving bands of 6-phosphogluconate dehydrogenase in skin tissues of melanoma patients, it could be of some diagnostic value. Our observation (unpublished data) shows that these 2 types of bands of 6-phosphogluconate dehydrogenase may differ only in their binding capacity to NADP+. We added NADP+ to some of our gel during the preparation prior to electrophoresis and found that the fast-moving bands move the same as the slow-moving ones. This indicates that when extra NADP+ is supplied, both isozymes bind with this cofactor and their electrophoretic mobility becomes the same.

We tried to relate the changes of the isozyme found in the tumor tissues with the age, sex, and stage of malignancy. The significance of these changes is not apparent at this time.

Further studies are under way to elucidate these findings and to determine their significance. Additional data and some in vitro studies are required for further characterization of isozyme variation in relation to melanoma.

REFERENCES


1438 CANCER RESEARCH VOL. 34
Isozyme Variations in Human Malignant Melanoma

Cancer Res 1974;34:1435-1438.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/34/6/1435

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.